THE BIOLOGY OF ASCIDIA NIGRA (SAVIGNY). I. SURVIVAL AND MORTALITY IN AN ADULT POPULATION

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Ascidia nigra is a solitary ascidian with a jet black test and measuring 10 to 12 cm. in length when fully grown. The colour of the test is due to pigment granules which migrate out through the test and form a thin layer at the surface where the pigment continuously sloughs off, together with some of the test substance. This process, which was first described by Hecht (1918) and has been confirmed by the writer, makes it nearly impossible for other sedentary organisms to settle and grow on the test. Unlike many other ascidians, therefore, *A. nigra* has a clean test throughout its life and this, combined with its conspicuous colouration, makes it an ideal animal for ecological study; against its background it stands out conspicuously and the history of individuals may be recorded easily.

The species is widely distributed throughout the warm waters of the western Atlantic ranging from Florida (25° N.) to Saõ Sebastian, Brazil (24° S.). It occurs in Bermuda (32° N.) (Van Name, 1945) and is recorded from the Red Sea, Gulf of Aden and Gulf of Guinea (Millar, 1958). Throughout the West Indies it is one of the commonest inshore shallow-water ascidians, usually confined to the sheltered waters of harbours and mangrove lagoons; it is only rarely found on coral reefs. In the harbours and lagoons it is found attached to mangrove roots, piers, pilings, buoys and ship bottoms from surface level to about 25 feet; it also occurs sometimes on the sea floor where a suitable hard substratum, such as a stone, enables it to settle. In general it is a primary coloniser, developing large populations on new or cleaned surfaces but ultimately being replaced by other organisms. The primary sessile community in Kingston Harbour, Jamaica, is dominated by algae (*Enteromorpha*), cirripedes (*Balanus amphitrite* Darwin), hydroids, serpulids and the ascidians [1] *A. nigra, Didemnum candidum, Diplosoma macdonaldi, Symplegma viride* and *Polyclinum constellatum*. This gives way by various stages to a climax community dominated by sponges (principally *Haliclona* sp., *Tedania ignis* (Duchassaing and Michellott) and *Mycale cecilia* deLaubenfels), anemones (*Aiptasia tagetes* (Duchassaing and Michellott)), ophiurans (*Ophiothrix angulata* Say) and the ascidians *Microcosmus exasperatus, Herdmania momus* and *Pyura vittata,* but in which *Ascidia nigra* is relatively rare. A detailed account of succession in this community will be published in a latter paper.

The present paper describes the history of a population of *Ascidia nigra* which settled and developed naturally on panels suspended in the sea at Port Royal in Kingston Harbour, Jamaica (Station C in Goodbody, 1961a). During the period of observation the population suffered a density-independent mortality following

[1] Nomenclature of ascidians is taken from Van Name (1945).

an influx of fresh water to the harbour; the details of this are discussed below and in the paper referred to above.

Methods

A raft (or pontoon) four feet square was moored 20 feet away from the sea wall at Morgan's Harbour, Port Royal, where the water is 23 feet deep. On each of the four sides of the raft two panels were suspended one below the other on galvanised chains so that the upper panel had its upper edge 4 feet below the water surface and the lower one 7.5 feet below the surface. The panels hung so that the settling surfaces were vertical in the water. In this way 8 panels were suspended from the raft, 4 at each vertical level.

Each panel was made of a rectangular sheet of ⅛-inch-thick "Tufnol"[2] 18 × 15 inches, bound along each side of each long edge by a thin strip of steel 1.5 inches wide. This reduced the area of "Tufnol" available for settlement to 18 × 12 inches. Both sides of each panel were available for settlement and were designated Front (F) and Rear (R) according to whether it faced out from the raft (F) or in towards the mooring chain (R).

The raft was moored by a single central chain and swivel so that it could turn horizontally about a central axis. Though undesirable from some points of view, this and the small size of the raft were essential parts of an attempt to minimise the risk of destruction by hurricanes.

The 8 panels were placed in position on August 1, 1957, and were not finally removed until May 6, 1960. At intervals the panels were removed from the raft and taken to a tank in the marine laboratory. The maximum time any panel was out of the water at any one time was one minute. In the laboratory the position of each specimen of *A. nigra* was plotted on a map of the panel and a colour photograph of each side of the panel was taken. From the photographs permanent maps were made and each animal designated by a serial number. In this way a permanent record was obtained of the history of each animal whose position was known on the map from its first appearance to its ultimate death or disappearance.

In the course of lifting and examining panels a total of five animals were accidentally killed. These have been excluded from the analysis of data except in Table II which is concerned solely with new settlements.

Colonisation and population growth

Table I shows a summary of the history of the population, and in columns 4 and 5 and in Figures 1 and 2 are shown the number of new animals appearing on the panels and the total population present. It will be seen that the majority of animals colonised before September 26, 1957 (57 days after the start of the experiment) and very few animals colonised after October 25, 1957. Newly settled *A. nigra* do not begin to develop the black pigment until about 20 days after metamorphosis and hence are only just visible to the naked eye as small black

[2] "Tufnol" is the trade name for a synthetic resin bonded fabric sheet conforming to British Standard No. 972: 1941. It is ideal material for experimental panels as it is the colour of dark wood and is completely unaffected by sea water or by boring animals as *Teredo* or *Limnoria*.

objects when 4 weeks old (unpublished observations). It is probable, therefore, that the majority of animals settled on the panels in the first 4 weeks but were not all visible when they were first examined on August 28, 1957. It is clear from these figures that once the primary colonisation is complete few new animals settle in the community. Elsewhere (Goodbody, 1961c) I have shown that *Ascidia nigra* breeds throughout the year in Jamaica, so that the fall-off in numbers

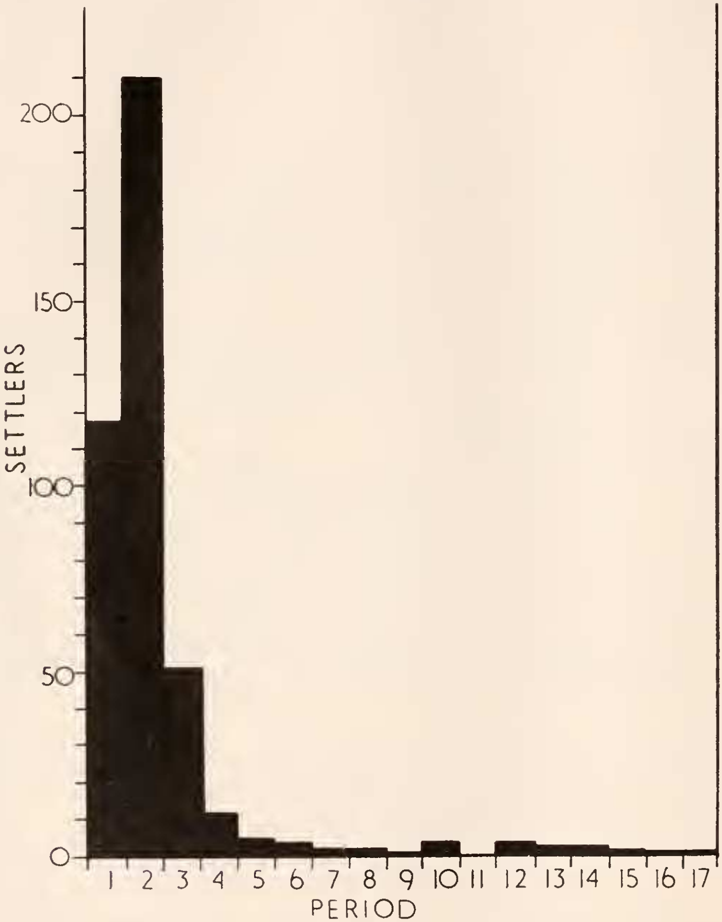

FIGURE 1. The number of new *Ascidia nigra* appearing on panels (settlers) at each inspection throughout the life of the population. The length of each period is not the same (see Table I).

of new animals appearing on the panels cannot be due to a seasonal drop in the number of larvae available. The fall-off in numbers must therefore be due to competition, either inter- or intraspecific, most probably the former. If intraspecific competition was important in determining settlement and growth of new individuals it would be expected that late settlers would tend to appear on panels with few other *A. nigra*.

Table II examines this possibility and analyses data for 27 new settlements between November 1, 1957, and October 3, 1958. It is clear from this table that there was no tendency for new settlements to occur on panels with few, as opposed to many, other *A. nigra*. It is more probable that the suppression of later colonisations is an effect of the whole community, in fact a combination of inter- and intraspecific competition. Most of the organisms on the panel are leptopel feeders competing for similar food to that of *A. nigra*, and any newcomer must have difficulty in obtaining sufficient food for growth. Furthermore, sponges may actually inhibit the development of other sessile organisms (Goodbody, 1961b).

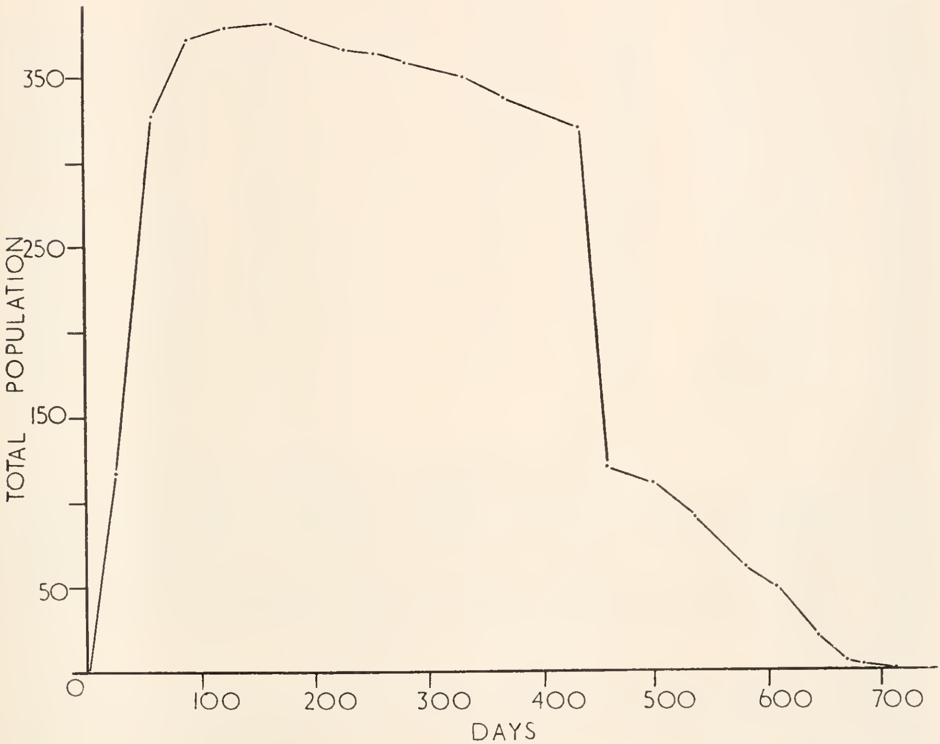

FIGURE 2. The total population of *Ascidia nigra* present on panels throughout the life of the population. The sharp drop after day 435 is due to fresh-water floods (see text).

Figure 2, which illustrates the growth of the total population, also illustrates the rapid rate of colonisation of the new panel. Subsequent to the initial rapid rise in population size, the population becomes stable for a long period, and since few new colonisations are taking place this indicates also a low mortality rate which is discussed in the next section.

The data outlined above, depicting *A. nigra* as a primary coloniser in sessile communities, are substantiated by numerous field observations of natural situations. In recent years several mass mortalities have occurred among sessile communities in Kingston Harbour (Goodbody, 1961a) and subsequently large areas of cleaned

TABLE I

Total settlement, mortality and mortality index. Mortality index is found by multiplying percentage mortality by $\frac{100}{no.\ days}$

Period	Date	Interval in days	Total population	No. new animals settling	No. animals dying	% Mortality	Mortality index
1.	28. 8. 57	28	117	117	—	—	—
2.	26. 9. 57	29	327	210	0	0	0
3.	25. 10. 57	29	372	50	5	1.5	5
4.	27. 11. 57	33	379	11	4	1.1	3
5.	8. 1. 58	42	381	4	2	0.5	1
6.	6. 2. 58	29	373	3	11	2.9	10
7.	12. 3. 58	34	366	1	8	2.1	6
8.	9. 4. 58	28	363	1	4	1.1	4
9.	6. 5. 58	27	358	0	5	1.4	5
10.	25. 6. 58	50	350	3	11	3.1	6
11.	30. 7. 58	35	337	0	13	3.7	11
12.	3. 10. 58	65	320	3	20	5.9	9
13.	27. 10. 58	24	120	2	202	63.1	263
14.	8. 12. 58	42	110	2	12	10.0	24
15.	13. 1. 59	36	90	1	21	19.1	53
16.	26. 2. 59	44	60	0	30	33.3	76
17.	26. 3. 59	28	49	0	11	18.3	65
18.	30. 4. 59	35	19	—	30	61.2	175
19.	27. 5. 59	27	4	—	15	78.9	292
20.	10. 6. 59	14	2	—	2	50.0	357
21.	7. 7. 59	27	0	—	2	100	370

surfaces became available for larval settlement on mangrove roots, piers, pilings, etc. *A. nigra* has always been one of the first colonisers of these surfaces, sometimes developing as dense clusters of several hundred individuals. Similarly, whenever new piles are driven this species develops in dense clusters in the early stages. However, as the sessile community develops on these surfaces, *A. nigra* is slowly replaced by dominants such as *M. exasperatus*, *P. vittata* and *H. momus*, together with sponges, anemones and lamellibranchs.

Why *A. nigra* should succeed only as a primary coloniser is not clear at present, but it is pertinent to point out that at all stages of its growth the siphons project

TABLE II

Settlement of new animals, in relation to density of A. nigra *already on panel, between 25th October 1957 and 3rd October 1958*

Panel density	No. of panels	No. of settlements	No. of settlements per panel
11–15	4	6	1.5
16–20	2	2	1.0
21–25	4	6	1.5
26–30	3	7	2.3
31–35	2	5	2.5
36–40	1	1	1

out into the water beyond the level of the remainder of the community, including other ascidians. This suggests that competition for food may be of paramount importance and that by keeping the siphons projecting it can tap the food supply before it reaches the other members of the community.

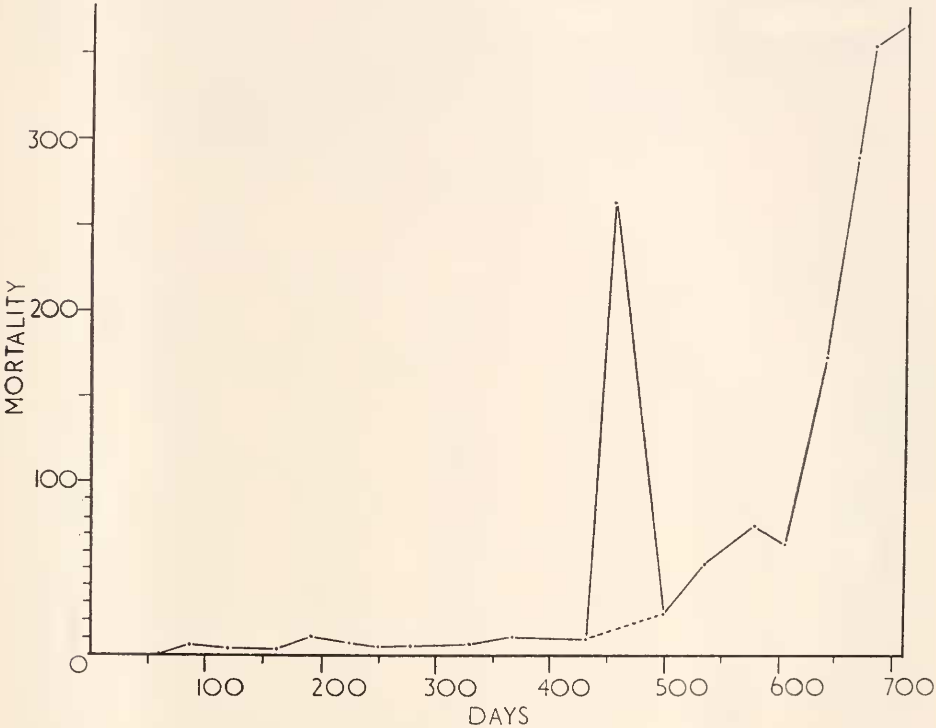

FIGURE 3. Mortality rate expressed as mortality index (see text). The dotted line connects the two periods before and after the fresh-water floods.

MORTALITY AND SURVIVAL

The intervals between successive inspections of the panels were not constant and so it is not possible to construct the usual form of life table. The succeeding analysis is therefore confined to two functions of the life table, mortality rates and survival. In place of the usual "mortality rate" (1000 qx) I have substituted a "Mortality Index" which may be expressed as

$$\frac{100}{I} \times \frac{100D}{P} = (I \times 10^{-1})\ 1000\ qx,$$

where I is the interval in days, D is the number of deaths during the interval and P the total population at the beginning of the interval. The Mortality Index provides a means of comparing the mortality rate over the whole life span of the population.

The mortality index for the whole population is shown in Table I and Figure 3. In calculating this function no allowance has been made for the small increment in the population subsequent to the establishment of the main population. It is clearly demonstrated here that from the time animals first appeared as small black ascidians, the mortality rate was low until the population was about 15 months old, after which it climbed rapidly until the last animal died when the population was 23 months old. The greatest life span attained by any one animal was an

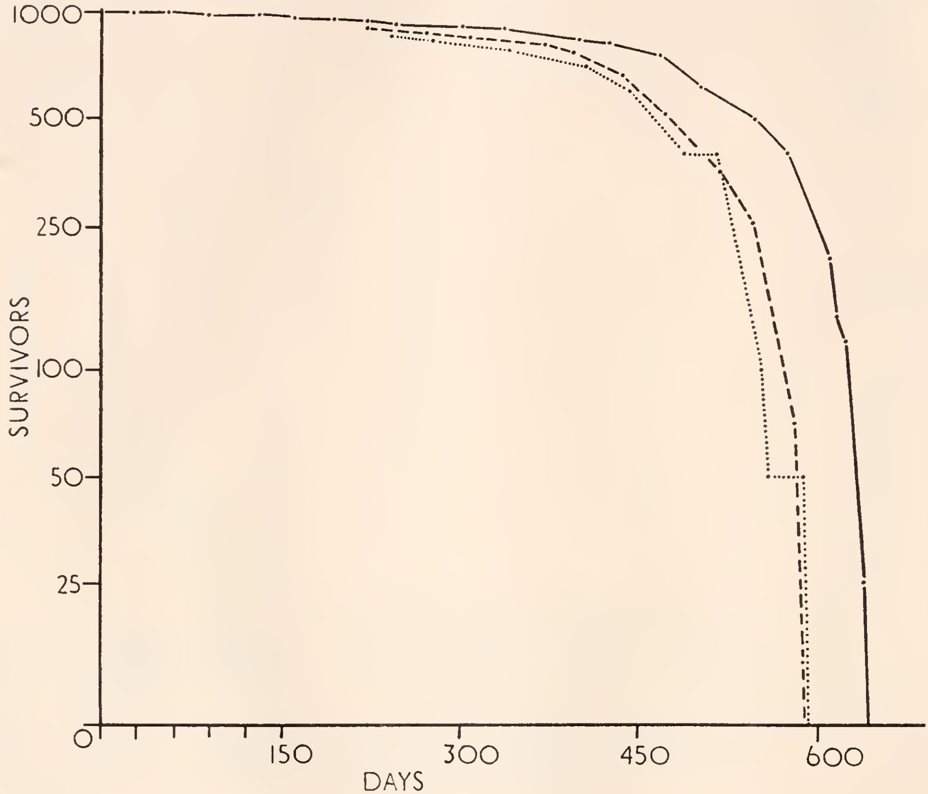

FIGURE 4. Corrected survival curves for three groups of ascidians which first appeared on the panels in August, September and October, 1957 (see Table III). •——————• : Group A, appearing in August. •- - - - - - - - - - - • : Group B, appearing in September. .... : Group C, appearing in October. For the sake of clarity curves B and C have not been carried back to the origin.

individual which appeared at the first inspection, hence settled in the first week of August, 1957, and died between May 27 and June 3, 1959. It must therefore have been between 94 and 96 weeks old (22 months) at the time of death.

The curves shown in Figures 2 and 3 are complicated by a density-independent mortality due to fresh-water floods occurring between October 3 and 27, 1958. Following heavy rain a layer of low-salinity water penetrated the harbour and caused extensive mortality among sedentary organisms (Goodbody, 1961a). On panels at the 4-foot level 86% of the *A. nigra* were killed and at the 7-foot level 33% were killed. Subsequent to this mass mortality the mortality curve climbs steadily upward, but there is no reason to believe that this is not normal or that it is in any way connected with the floods. Data from 18 animals in a pilot experiment run in 1956–58 show that 50% had died in 12 months and all were dead after 85 weeks (19½ months).

Table III

Survival rate (1x) in three separate groups of A. nigra *showing the observed rate and the recalculated rate for a population free from density independent mortality (see text). Group A settled in August, 1957, B in September and C in October. Figures in parentheses show actual number of animals in each group at outset. For dates corresponding to period numbers see Table I. Periods added to this table are 18a: 6. 5. 59, 18b: 13. 5. 59, 18c: 20. 5. 59, 19a: 3. 6. 59*

Period No.	Observed Survival (1x)			Recalculated Survival (1x)		
	Group A (117)	Group B (210)	Group C (50)	Group A (117)	Group B (210)	Group C (50)
1.	1000	—	—	1000	—	—
2.	1000	1000	—	1000	1000	—
3.	1000	976	1000	1000	976	1000
4.	991	967	980	991	967	980
5.	991	957	980	991	957	980
6.	974	938	920	974	938	920
7.	974	919	920	974	919	920
8.	957	914	920	957	914	920
9.	940	900	920	940	900	920
10.	915	876	860	915	876	860
11.	897	848	840	897	848	840
12.	838	810	780	838	810	780
13.	350	262	300	811	769	755
14.	325	224	280	753	658	704
15.	265	176	240	614	517	603
16.	179	124	160	500	364	402
17.	145	90	160	405	264	402
18.	77	24	40	215	70	100
18a.	51	5	20	142	15	50
18b.	43	0	20	120	0	50
18c.	9	—	20	25	0	50
19.	9	—	20	25	0	50
19a.	0	—	20	0	—	50
20.	0	—	0	0	—	0

Since settlement of young animals goes on over a period of three months with small increments in later intervals, the population being dealt with cannot be considered as homogeneous in age structure. The information in Table I is therefore of limited interest only as a history of the entire population. It is more informative to consider separately the life table data for each of the three groups of animals settling in the first three months and to see if any differences exist between them. This is accomplished by a comparison of survivorship (1x) data and is shown in Table III and Figure 4 for the groups of animals which first appeared on panels in August, September and October, 1957.

Table III has been constructed so as to show the actual survival, and calculated survival data in which the effect of the density-independent mortality has been eliminated. To calculate the latter it has been assumed that the mortality index between October 3 and 27, 1958, would normally have been the mean of that prevailing in the periods immediately preceding and succeeding it; from this a value for the percentage mortality in period 13 has been calculated for each of the three groups. By substituting this value in place of the observed mortality in period 13 the 1x values for periods 13–21 can then be re-calculated to give a picture of the survival curve for a population free from density-independent mortality.

Figure 4 shows corrected survival curves for the three populations. In constructing this figure it has been assumed that population A settled on August 1, B on August 28, and C on September 26, and the ordinate plots the number of days since settlement. In this way the three survival curves can be directly compared. This curve can be only an approximation to the true survival curve, but it is probably reasonably close. The obvious sources of error are in the calculation of the per cent mortality for period 13 and in assuming that all the animals in a group settled at the chosen dates. Furthermore, this does not include mortality in the first 28 days and is only a survival curve for animals of 28 days and over.

Two features of interest are apparent in Figure 4. First the survival curve in each case is of the "negative skew" rectangular type (Deevey, 1947) in which few deaths occur until very near the maximum length of life and most of the animals die in a short space of time near the end. This suggests senescence and physiological longevity and is discussed further below.

Secondly, although the form of the curve is similar in all three groups the first settlers appear to have lived for longer than the other two groups. The 50% level was reached after 550 days in Group A, 480 in Group B and 465 in Group C. There are three possible explanations for this difference. (1) The population might really be homogeneous, all having settled in the first week of August, and the early development of some of them may have been greatly retarded by, for example, food shortage, so that they appeared very much later than the others. This possibility, though real, can probably be ruled out. The maximum error that could have arisen this way is 58 days, while the difference in longevity is of the order of 85 days. (2) The first settlers may be genuinely more successful than later settlers and consequently live longer. (3) The sharp drop in the curve may be the result of a change in the environment and be ecological mortality and not senescence.

It is not possible at present to decide definitely between these two latter possibilities but in assessing them the following points may be considered. Life spans of 18–20 months have been calculated for several temperate water species of ascidians by Millar (1952, 1954) and Sabbadin (1957, 1958), and animals in a pilot experiment with *A. nigra* in 1956–58 had a total life span of 19–20 months. This supports the contention that the curve may be natural and senescent. On the other hand I have shown elsewhere (Goodbody, 1961c) that the climax sponge/anemone/ophiuran community inhibits the development of the early stages of the primary colonising community, possibly as a result of toxins produced by sponges or by competition for food. This "influence" might extend to the adult population of *A. nigra* and be responsible for its death. In support of this we may note that the final death of all three populations occurred at approximately the same time (Table III), and that this coincided with the time when the climax community was reaching full development. This problem can be resolved only by further experiment.

While there is a high rate of survival in populations settling in the first few months, the same does not appear to be true for animals which settle at later times. In Table IV the survival of animals to October 3, 1958 (*i.e.,* immediately before the floods) is shown for animals settling at different periods. There is a considerable and significant difference between those settling in August, September and October and those settling from November to June. Here is further evidence that *A. nigra* can survive only as a primary coloniser in the community, and that new individuals do not appear in the community because they cannot compete in it.

TABLE IV

Showing different rates of survival in groups of Ascidia nigra *settling at successively later stages. Survival has been taken to 3. 10. 58 (approximately one year) as density-independent mortality occurred on 6. 10. 58*

Settlement date	Number settling	Number surviving 3. 10. 58	% Surviving
Aug., 1957	117	98	84
Sept., 1957	210	170	81
Oct., 1957	50	39	78
Nov., 1957	11	5	45.5
Dec., 1957 to June, 1958	12	5	42

I know of no active predator on the adult ascidian and it appears that the test substance is distasteful. Fishes in an aquarium will eat the body tissue of an *Ascidia nigra* but examine and reject the test substance if that is fed to them. A small amphipod, *Erichthonius brasiliensis* (Dana) is common on the test and in and around the siphonal margin, but this is probably a true commensal arrangement. Several copepod commensals occur in the branchial sac and also a large pea-crab, *Pinnotheres moseri* Rathbun. The incidence of this crab in animals is very variable but there seems to be never more than one in any ascidian. There is no evidence that it harms the ascidian and all infected animals have appeared normal and healthy.

Externally a galatheid crab and the ophiuran, *Ophiothrix angulata,* are commonly found wandering on the test but have probably no effect other than to help keep the test clean. A large xanthid crab (*Menippe nodifrons* Stimpson) occasionally makes tunnels around the base of sessile organisms in this community and in a later experiment was suspected of actually dislodging some ascidians from a panel, but this is rare.

DISCUSSION

Previous studies of the life cycle of solitary ascidians have been published by Millar and Sabbadin, both of them concerned with temperate-water species. Millar (1952) studied *Ascidiella aspersa* and *Ciona intestinalis* in the Clyde, Scotland, and found the pattern of the life cycle of both species to be fairly similar. There is a single breeding season in summer, involving several generations of larvae; and the young animals grow until late autumn when growth ceases. In the following spring growth re-commences and these animals form the breeding stock for the subsequent summer spawnings. This adult population died off in the following winter, thus having a total life span of 12–18 months. In a later paper Millar (1954) studied populations of *Dendrodoa grossularia* from the Clyde and from Essex, England. This species has a similar form of life cycle, living for from 18 to 24 months, but in Essex has two distinct breeding seasons in each summer. Sabbadin (1957, 1958) studied *Ciona intestinalis, Molgula manhattensis* and *Styela plicata* in Venice, Italy. Breeding is continuous from early spring to late autumn in all three species. Animals metamorphosing in early spring may breed during the ensuing summer and have completed their growth within the year. Later settlers cease growing during winter, and do not complete their growth and breed until the following spring. The total life span is reported to be "about one year" (Sabbadin, *loc. cit.*).

Except for the similarity in total life span, the pattern of annual cycle in these temperate-water species differs from that of *Ascidia nigra*. Whereas the former have annual breeding seasons, *A. nigra* breeds throughout the year so that the population is composed of animals of all ages. Present information suggests that *A. nigra* commences breeding when it is about 85 days old and thereafter may spawn at intervals of about 60 days.

Growth in the temperate-water species is arrested during the winter months and according to Sabbadin (1957) is definitive. However, this author studied ascidian populations by means of monthly samples and his observations on growth are based entirely on these samples. From such data it is not possible to say with certainty that growth is not continuous throughout life. Unpublished data on *A. nigra* suggest that in Jamaica this species may continue to grow throughout life though at a progressively retarded rate. This point is important in relation to senescence and the problem of why ascidians die.

Life tables for other animals have been extensively reviewed by Deevey (1947) and by Allee *et al.* (1949). The point of interest here is in the type of survival that is illustrated. The curve shown in Figure 4 is typical of a population which exhibits senescence (Comfort, 1956) and very little ecological mortality. This raises again the question of why ascidians die. It has been mentioned earlier that the decline in the ascidian population is coincident with the rise of the climax sponge community. Experiments are now in progress to try and determine whether one is dependent on the other or whether the ascidians die for physiological rather than ecological reasons. In the studies by Millar and Sabbadin (*loc. cit.*) populations died over the winter months when water temperatures were cool. In these areas declining temperature may be the cause of, or hasten, the onset of death and thus obscure the incidence of senescence. In Jamaica, water temperatures vary about 6° C. throughout the year (Goodbody, 1961c) and are unlikely to be concerned in any way with death.

Finally it should be emphasized again that these data deal only with ascidians from the twenty-eighth day of life onwards and more drastic mortality is to be expected in the first four weeks. Data on this period are at present accumulating and will form the subject of a later paper in the series.

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Summary

1. A "marked" population of *Ascidia nigra* has been followed throughout life from their first appearance to the time of death.

2. Mortality in the first four weeks after metamorphosis has not been studied. Thereafter few animals die until they are 18 months old and all are dead at 22 months old.

3. A mass mortality due to fresh-water floods occurred in the period of observation. A corrected survival curve has been calculated for a situation in which this did not occur. This suggests that senescence may occur.

4. *A. nigra* is a primary coloniser. When colonising new surfaces, the earliest colonisers survive longer than the later ones. Very few animals can colonise after the primary sessile community is three months old.

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